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Wildlife Toxicity Assessment for 1,3,5-Trinitrohexahydro-1,3,5-Triazine (RDX)

JULY 2002

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

USACHPPM Document No: 37-EJ-1138-01H
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Readiness Thru Health

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Acknowledgements

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When referencing this document use the following citation
Salice, C.J. and G.Holdsworth. 2001. Wildlife Toxicity Assessment for 1,3,5-Trinitrohexahydro-1,3,5-Triazine (RDX). U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) Project Number 39-EJ1138-01B, Aberdeen Proving Ground, Maryland, May 2002.

Table of Contents

Wildlife Toxicity Assessment for RDX

CAS No.121-82-4 **DRAFT**

1. INTRODUCTION

The explosive 1,3,5-Trinitrohexahydro-1,3,5-Triazine (CAS No. 121-82-4) is more frequently known as RDX (Royal Demolition Explosive). RDX is an explosive chemical that has found widespread application in detonators, grenades, bombs and a variety of other military ordnance. Structurally, the compound is a trinitro-substituted triazine with the empirical formula, $C_3H_6N_6O_6$. In addition to RDX, other synonyms include: 1,3,5-triaza-1,3,5-trinitrocyclohexane, hexahydro-1,3,5-trinitro-1,3,5-triazine, cyclotrimethylenenitramine, hexogen, cyclonite, among others (ATSDR, 1995). The importance of RDX as an environmental contaminant is related to its widespread distribution at and around military sites and its potential toxicity to wildlife and other ecological receptors. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of RDX on wildlife and reports toxicity reference values (TRVs) for RDX. The TRVs are intended to serve as protective exposure standards for wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254 (TG254), the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were searched electronically August 23, 2000, using Dialog® to identify primary reports of studies and reviews on the toxicology of RDX. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. All available abstracts of articles selected as potentially relevant to TRV development were further evaluated using criteria outlined in TG254 (USACHPPM, 2000). For RDX, 19 articles were marked for retrieval from 31 initial hits. Details of the search strategy and the results of the search are documented in Appendix A.

In addition to searching the Dialog Inc. database, a number of U.S. Army reports were identified in the Defense Technical Information Center (DTIC). Secondary references and sources of information on RDX included an Agency for Toxic Substances and Disease Registry (ATSDR) *Toxicological Profile for RDX* (ATSDR, 1995), the National Library of Medicine's Hazardous Substances Databank (HSDB, 2000), the U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2000) and Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997).

2.2 Environmental Fate and Transport

Military grade RDX (containing about 10% high melting explosive (HMX) by weight) has been a widely used explosive since the early years of World War II, when it began to either replace or supplement trinitrotoluene (TNT) as the primary ingredient in shells, bombs and detonators. Although the compound is currently manufactured only at the Holston Army Ammunition Plant (AAP) in Kingsport, Tennessee, a pattern of manufacturing and assembling practices has resulted in its release to the environment in considerable amounts at this and other sites, either as a single compound or mixed with other explosives. Talmage et al. (1999) reported that concentrations of up to 30 mg/L RDX had been detected in groundwater at Milan AAP, while surface water impoundments and associated sediments at this facility also displayed concentrations in the ppm range. Soil concentrations of up to 13,900 mg RDX/kg are listed by Talmage et al. (1999) for this and other military sites. Physicochemical properties of RDX relevant to its environmental fate and transport are listed in Table 1.

Table 1. Summary of Physical-Chemical Properties of RDX

Molecular weight	222.26
Color	White
Physical state	crystalline solid
Melting point	205–206 °C
Boiling point	decomposes
Odor	no data
Solubility Water	38.4 mg/L; slightly soluble in methanol, ether, ethyl acetate, glacial acetic acid
Partition coefficients:	
Log K_{ow}	0.87
Log K_{oc}	0.84–2.2
Vapor pressure at 20 °C	1.0×10^{-9}, 4.0×10^{-9} mm Hg
Henry's Law constant	1.2×10^{-5} atm.m3/mole
Conversion factors	1 ppm = 9.1 mg/m^3
	1 mg/m^3 = 0.11 ppm

Sources: ATSDR, 1995; Talmage et al., 1999; HSDB, 2000

RDX has an estimated vapor pressure of $1–4 \times 10^{-9}$ mm Hg at 25°C, a low value implying that partitioning to air is unlikely. Furthermore, the compound is soluble only to a limited extent in a number of common organic solvents and in water (38.4 mg/L at 20–25°C). However, despite its limited solubility, the compound has been detected in both surface water and groundwater (see Talmage et al. 1999 for review). Hovatter et al. (1997) and Talmage et al. (1999) also present RDX soil concentration data from other studies for a number of AAPs, depots and arsenals.

Photolysis is a potentially important process for degrading RDX, since the compound can absorb ultraviolet light strongly at wavelengths between 240 and 250 nm. In addition, biodegradation of RDX has been demonstrated under anaerobic conditions in the presence of a number of microbial isolates and mixed cultures, with total degredation in 5 days or less. Thus, when RDX was incubated in an anaerobic test system containing sewage sludge and mixed cultures in nutrient broth, the disappearance of RDX was accompanied by the formation of a range of metabolites including hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), hydrazine, 1,1-dimethyl-hydrazine, 1,2-dimethyl-hydrazine, formaldehyde and methanol (McCormick et al., 1981, 1984).

Studies have shown that plants are able to absorb RDX from soil and to a lesser degree from irrigation water. Radio-labeled RDX accumulated in bush bean grown on RDX amended soil with the highest concentration of RDX in the seeds followed, in order of decreasing concentration, by leaves, stems, roots and pods (Cataldo et al., 1990). Concentration ratios were on the order of 20 to 60 for seeds and leaves, which suggests an efficient uptake mechanism and high plant mobility. Analysis of bush bean grown in RDX amended hydroponic solution showed that approximately 23 and 50% of the rabio-label present in the root and leaves, respectively, was parent RDX after a 7-day exposure (Harvey et al., 1991). The efficiency of RDX absorption varies with species and is inversely proportional to organic matter content of the soil. Studies on uptake of RDX from spiked irrigation water showed a lower uptake of RDX by tomato, bush bean, corn, soybean, alfalfa, lettuce and radish (Checkai and Simini, 1996). Concentrations of RDX in the plants were less than that of the irrigation water. Thus, research to date indicates that plant uptake of RDX is highest from RDX contaminated soils and, importantly, that RDX in plants can be a potential exposure route for herbivorous terrestrial wildlife.

2.3 Mammalian Toxicity

2.3.1 Mammalian Toxicity - Oral

2.3.1.1 Mammalian Oral Toxicity – Acute

Dilley et al. (1978) conducted a number of toxicological tests on TNT, RDX, and a mixture of the two compounds styled "LAP" (for load, assembly and pack wastewater), employing mixtures of each compound in corn oil that were administered to the test animals by gavage. Mortality and clinical parameters were observed on all survivors for 14 days prior to termination. The acute oral LD_{50} for RDX in male Sprague-Dawley rats was 71 mg/kg-day compared to values of 1320 and 574 for TNT and LAP, respectively. Dilley et al. (1978) reported respective acute oral LD_{50}s of <75 and 86 mg/kg-day for RDX in male and female Swiss-Webster mice, compared to 660 mg/kg-day for TNT in either sex of mouse and 947 and 1131 mg/kg-day for LAP in male and female mice, respectively.

Data on the acute oral lethality of RDX in experimental animals were provided also by Cholakis et al. (1980), who determined LD_{50}s in male and female F344 rats (10/sex/group) and B6C3F1 mice (5/sex/group) given single doses of the compound by gavage in either 1% aqueous methylcellulose (rats) or a mixture of 1% methylcellulose and 1% polysorbate 80 (mice). Broadly consistent with the findings of Dilley et al. (1978), Cholakis et al. (1980) derived a combined (male/female) acute oral LD_{50} of 118.1 mg RDX/kg-day in F344 rats and 80.3 mg/kg-day in the mice. Overall, the acute lethality data on RDX of Dilley et al. (1978) and Cholakis et al. (1980) have yielded lower values than the LD_{50} of 200 mg/kg-day obtained for the compound in the earlier studies of von Oettingen et al. (1949).

In addition to acute oral lethality, single dose experiments with RDX have been used to determine the toxicokinetic behavior of the compound in experimental animals. For example, Schneider et al. (1977) administered 100 mg RDX/kg by gavage to Sprague-Dawley rats (n = 70, sex not specified) and Pittman-Moore miniature swine (n = 10 female) and monitored the partitioning of the compound between feces, urine and the major organs and tissues. Only a small amount of RDX (less than 3%) was recovered in the feces of the rats, suggesting that the bulk of the material had been transported across the gastrointestinal absorption barrier. When 50 mg/kg ^{14}C RDX was administered to the rats, most of the radioactivity was found in the liver and urine after 24 hours, with further partitioning to other parts of the body during the next three days. Overall, 43% of the radioactivity was expired as $^{14}CO_2$.

Inferential support for the concept of the liver as a major catabolic site for RDX is provided by French et al. (1976) who, in a meeting abstract, reported profound ultrastructural changes in the liver of rats (strain, sex, number unstated) as a result of oral administration of a single dose of 100 mg RDX/kg. Among other membrane perturbations, the smooth endoplasmic reticulum was highly proliferated after 48 hours, possibly indicating the induction of the mixed function oxidase system. By contrast, ultrastructural changes to the kidney due to RDX were minor and inconsistent.

Although the liver appears likely to be the primary site of RDX catabolism, the compound or another pharmacologically active metabolite of RDX has the capacity to induce neurotoxicological responses in male and female Sprague-Dawley rats. Thus, in the acute section of a multiphase study, MacPhail et al. (1985) administered single gavage doses of up to 50 mg RDX/kg in 2% carboxymethylcellulose and observed overall decreases in such responses as startle-response amplitude, startle-response latency, figure-8 maize motor activity, conditioned flavor aversions and schedule-controlled responses.

2.3.1.2 Mammalian Toxicity – Subacute

Ferguson and McCain (1999) conducted a 14-day, subacute study on the oral toxicity of RDX to the white-footed mouse, *Peromyscus leucopus*. Ten male and ten female mice were in each of five RDX exposure groups. RDX was mixed with feed in concentrations of 0.00, 0.05, 0.10, 0.20, 0.40 and 0.80 mg RDX/g feed which corresponded to oral doses of 0, 8, 16, 31 and 59 mg RDX/kg body weight/day in males and 0, 8, 15, 32 and 68 mg RDX/kg body weight/day in females. Exposure continued until day 14 at which point, mice were euthanized by carbon dioxide asphyxiation. Data on feed consumption, body weight, organ weight, organ-to-body weight ratio and organ-to-brain weight ratios were collected and statistically analyzed. Blood samples were obtained and used for hematological and clinical chemistry analyses, however, the analyses could not be conducted so the data were unavailable. After examining tissues for gross pathological lesions, the liver, kidney, spleen, brain, thymus, and testes were collected and weighed from half of the animals in each group and submitted for histological examination. The same tissues, minus the spleen, thymus, and brain from the remaining animals were frozen and analyzed for biochemical parameters.

Results indicated very little compound-induced toxicity. In part, the authors attributed the lack of anticipated toxicity of RDX to *P. leucopus* to a higher metabolic rate and faster food transit time, which may increase the resiliency of this species compared to other *Mus* species. Mice exposed to RDX did show increased ovary, ovary-to-brain and ovary-to-body weights for the groups fed 0.05, 0.20 and 0.40 mg RDX/g feed. The effect, however, was not considered biologically significant since there was not a dose-response and the findings were unsupported by histological analyses. Similarly, liver weights were increased for females from the 0.05 and 0.20 exposure groups. Again, the finding was not considered biologically significant or compound related. Females in the two high dose groups showed an increase in spleen weight and spleen-to-brain weight ratios. Histopathological analyses did not reveal any treatment-related effects although the significant weight changes in the high dose group suggest possible RDX-induced toxicity. A potential NOAEL based on the weight change in the spleen is 16 mg RDX/ kg bw/day while a potential LOAEL is 31 mg RDX/kg bw/day.

2.3.1.3 Mammalian Toxicity – Subchronic

Mammalian species that have been used as models for testing the subchronic toxicological impact of RDX include beagle dogs, cyanomolgus (rhesus) monkeys, Sprague-Dawley and F344 rats and B6C3F1 and Swiss-Webster mice. Litton Bionetics (1974a) exposed three beagles/sex/group to 0.1, 1 or 10 mg RDX/kg-day as a dietary additive for 90 days. Urinalysis was carried out after four weeks, eight weeks and at term, along with clinical chemistry and hematological determinations in blood samples collected at the same intervals. All survivors were subjected to a gross necropsy at term, organ weights were recorded, and histopathological comparisons of the brain, thyroid, lungs, heart, liver, spleen, kidney, adrenals, stomach, small intestine, and bone marrow were made between the high-dose and control groups. However, no abnormal findings in any measured parameter were noted at the doses chosen for the study.

Similarly, in another study by Litton Bionetics (1974b), the same exposure duration and dose levels (by gavage) as for the dogs showed the subchronic effects of RDX to be comparatively benign in rhesus monkeys. The appearance of increased numbers of degenerate or necrotic megakaryocytes in sections of bone marrow from some high-dose monkeys led to a dose of 1.0 mg/kg-day as a no observed adverse effect level (NOAEL) for this study. However, elevated numbers of megakaryocytes appeared in one of the three control animals examined for this feature, thereby suggesting the effect may not be compound-related.

Brown (1975) reported a study on rats (number and strain unstated) in which RDX was administered in the diet at doses of 0, 0.3, 2.5, 6.5, 15, 50 or 100 mg/kg-day for 12 weeks. Increased levels of RDX in the blood in response to all doses except the lowest were associated with increases in the specific activities of brain monoamine oxidase and cholinesterase and in the capacity of excised brain tissue to take up oxygen. Since these effects were negligible at the lowest dose, 0.3 mg/kg-day was chosen as a subchronic NOAEL for RDX.

The toxicological studies on RDX reported by Cholakis et al. (1980) featured 90-day studies in F344 rats and B6C3F1 mice in which 10 animals/sex/group were exposed to RDX in feed at doses of 0, 10, 14, 20, 28 and 40 mg/kg-day. In a supplemental study, additional mice were exposed to 0, 40, 60 and 80 mg/kg-day for 2 weeks and then to 0, 320, 160 and 80 mg/kg-day, respectively, for the final 11 weeks of the investigation. A suite of toxicological endpoints were monitored, including clinical signs, body weights and food consumption, clinical chemistry and hematological parameters, gross pathology and histopathology.

In the rats, there was a reduction in body weight gain in the high-dose males concomitant with a reduction in food consumption. In addition, sporadic though possibly compound-related hematological changes were noted, including a reduction in hemoglobin and hematocrit in high-dose males and males receiving 28 mg/kg-day after 30 and 60 days. Reticulocytes and platelets were increased in high-dose males after 90 days. There were few if any changes in clinical chemistry parameters, gross pathology or histopathology in the rats receiving RDX, findings that, taken together, suggest a NOAEL of 20 mg/kg-day based on the hematological changes.

The absence of any compound-related toxicological consequences of the same doses of RDX in exposed mice led to a supplemental study in which a number of sporadic responses were observed. For example, a number of clinical signs were evident across the groups, with marked hyperactivity among the males. Four of 10 high-dose males and 2/12 high-dose females died during week 11 of the study. Perhaps the most consistent treatment-related changes were observed at gross necropsy where dose-dependent and statistically significant increases in absolute and relative liver weights were observed in both sexes of mice. These changes appeared to be associated with the onset of hepatocellular vacuolization and other histopathological liver lesions, supporting the designation of a NOAEL at the time-weighted average mid-dose of 145 mg/kg-day.

Levine et al. (1981) conducted a study similar to the 90-day study in F344 rats reported by Cholakis et al. (1980), but with dose levels extending to 600 mg/kg-day. At 600 mg/kg-day, most of the subjects developed tremors and convulsions followed by death. Less severe toxicological responses were evident at the lower dose levels, including a concomitant reduction in body weight gain and food consumption in males receiving 100 mg/kg-day. Among the compound-related clinical chemistry changes was a dose-dependent reduction in plasma triglycerides that was statistically significant at 30 mg/kg-day and above. Increased relative liver weight in females receiving 100 mg/kg-day justified the choice of 30 mg/kg-day as a subchronic NOAEL for RDX in this strain of rat.

The report of acute neurological effects of RDX in male Sprague-Dawley rats had a subchronic component in which animals were gavaged for 30 days with 0, 1, 3 or 10 mg RDX/kg-day in 2% aqueous carboxymethylcellulose (MacPhail et al., 1985). Neurotoxicological tests were carried out before the onset of dosing and then on days 16 and 31. However, no significant effects of RDX were observed at any of the dose levels.

Dilley et al. (1978) investigated the subchronic oral toxicity of a 1.6:1 mixture of TNT and RDX (LAP) in dogs, rats and mice. The subchronic toxicity of TNT, but not RDX was evaluated in the study as well. Generally, the authors concluded that the results suggested that TNT dominated the toxicity of the LAP mixture. Similar to the studies of Dilley et al. (1978) on LAP, Levine et al. (1990) reported a 90-day dietary study in 10 F344 rats/sex/group in which the toxicological effects of mixtures of TNT and RDX ("composition B") were evaluated. In this study, the authors concluded that many of the toxicological effects of each explosive individually were actually antagonized by the presence of the other compound.

2.3.1.4 Mammalian Oral Toxicity – Chronic

The first study to examine the chronic toxicity of RDX in experimental animals was that of Hart (1976), who administered the compound as a dietary supplement to 100 Sprague-Dawley rats/sex/group for 104 weeks. The stated RDX levels of 0, 1.0, 3.1 and 10 mg/kg have been interpreted by the IRIS compilers (U.S. EPA, 2000) and other reviewers (Talmage et al., 1999) as referring to doses in mg/kg (body weight)-day, though ambiguities in the study report suggest possibly that the above values might refer to the concentrations of RDX in feed. If this were the case, the actual dose levels would have been at least an order of magnitude lower than those normally assumed for this study, and possibly explain why, out of a full suite of clinical chemistry, hematology, urinalysis, gross pathology and histopathological examinations, few if any compound-related changes were observed. However, as it stands, the data point to a NOAEL of 10 mg/kg-day for RDX, the highest dose tested.

Levine et al. (1983) reported on the chronic toxicity of RDX in 75 F344 rats/sex/group exposed to the compound in feed in amounts equivalent to doses of 0, 0.3, 1.5, 8 or 40 mg/kg-day for a total of 2 years. Clinical signs were observed twice daily and food consumption and body weights were monitored weekly up to test week 14 after which, they were monitored biweekly. Ophthalmic examinations were carried out on subjects during weeks 2, 25, 51, 76 and 103. Blood samples were taken at weeks 13, 26, 52, 78 and 104 for clinical chemistry and hematological determinations. Interim sacrifices of 10 rats/sex/group were carried out at weeks 27 and 52. At these points and at term, animals were subjected to a gross pathological examination. Samples of a wide range of organs and tissues were preserved by chemical fixation. Tissues from animals in control and high-dose groups were examined histopathologically, along with sections of brain, gonads, heart, liver, kidney, spleen, and spinal cord from all dosed groups.

Most rats receiving 40 mg RDX/kg-day died during the treatment period, many displaying profound clinical signs such as tremors, convulsions, hyperactivity, and discolored/opaque eyes. Body weight gain was also reduced in this and the intermediate-dose group, a change potentially associated with reduced food consumption. High-dosed rats had reduced RBC counts, hemoglobin concentration, and hematocrit, while the platelet count was increased in intermediate-dose males, however, these hematological parameters fell within normal ranges (Wolford et al., 1986). There were some fluctuations in clinical chemistry parameters, including relative decreases in plasma cholesterol and triglycerides and in the activity of serum glutamate-pyruvate transaminase. High-dose females displayed an increased incidence of cataracts at week 78 and week 104. Organ weight changes were noted, in particular, an increase in the relative weights of liver and kidneys in both sexes of high-dose rats and a reduction in testis weights of high-dose males. Also, observations indicated toxic effects in the spleen as early as 6 months into the study. After 2 years, the appearance of a hemosiderin-like pigment in the spleen was evident in all dose groups from 1.5 mg/kg-day and up. This finding points to a NOAEL of 0.3 mg/kg-day, a value that was used as such by the IRIS compilers to derive a human health reference dose of 3×10^{-3} mg/kg-day (U.S. EPA, 2000).

A similar study to that described above was conducted by the same researchers on B6C3F1 mice in an experiment in which 85 animals/sex/group were exposed via diet to RDX at concentrations approximating doses of 0, 1.5, 7, 35 and 100 mg/kg-day (Lish et al., 1984). The high-dose level, 175 mg/kg-day, had been lowered during the course of the experiment due to high mortality. Reduced body weight gain was noted in both sexes of high-dose mice, although food consumption was comparatively unaffected. Hematological parameters showed little change, although hematocrit and hemoglobin concentrations were reduced in high-dose females at an interim time point. Hypercholesterolemic and hypertriglyceridemic effects of RDX were observed, the former parameter displaying marked dose-response. A number of gross pathological and histopathological effects of RDX were evident in the mice, including increased relative liver and kidney weights in high- and intermediate-dose animals. Histopathological changes at the 2-year time point included degeneration of the testes in high- and intermediate-dose males, suggesting a NOAEL of 7 mg/kg-day for this response. Other important histopathological effects of RDX included a dose-dependent increase in the incidence of hepatocellular adenomas and carcinomas in the liver of females.

2.3.1.5 Mammalian Oral Toxicity – Other

Schneider et al. (1978) followed their acute studies on the toxicokinetics of RDX in Sprague-Dawley rats with subchronic studies in which the compound was administered either in drinking water or by gavage at 20 mg/kg-day for up to 90 days. Some animals were also exposed via drinking water to saturated unlabeled or ^{14}C-labeled RDX. The results pointed consistently to the relative inability of the compound to accumulate in the plasma or tissues. Overwhelmingly, the compound was released to the urine or as $^{14}CO_2$, with lesser amounts in the feces and carcass.

Angerhofer et al. (1986) investigated the teratological potential of RDX in pregnant Sprague-Dawley rats. In a pilot study, six pregnant rats/group were given 0, 10, 20, 40, 80 or 120 mg/kg by gavage in gum acacia on gestation days (GD) 6–15, and the parameters measured at GD 20 included the numbers of viable fetuses, nonviable fetuses, resorptions, implantations, and corpora lutea. Fetal parameters included weight, size, sex, and the incidence of external malformations and visceral abnormalities. The lowest dose inducing maternal toxicity in the pilot study (20 mg/kg-day) was chosen as the highest dose in the main part of the study. In the main study, 25 pregnant rats/group were given 0, 2, 6 or 20 mg/kg by gavage in gum acacia on gestation days (GD) 6–15. 31% of females receiving 20 mgRDX/kg died in the main study. For the survivors, there were few changes in reproductive parameters compared to controls and no compound-related anomalies among the teratological findings. The authors suggested a dose of 2 mg/kg-day as a lowest observed adverse effect level (LOAEL) for the reductions in fetal size that were evident at the lowest dose tested. Inspection of the statistical results suggested that the original analyses may have been suspect. Statistical reanalysis of the data indicated that fetal size was significantly affected only at the highest dose, 20 mg/kg-day. Hence, the revised LOAEL is 20 mg/kg-day and the NOAEL is 6 mg/kg-day.

Reproductive toxicity and teratological studies have also been conducted by Cholakis et al. (1980), who administered 0.2, 2 or 20 mg RDX/kg-day by gavage to pregnant female F344 rats between GDs 6–19 and to New Zealand white rabbits between GDs 7–29. At sacrifice, the uteri were examined for live fetuses and resorptions, while the fetuses themselves were examined for skeletal abnormalities and visceral perturbations. Food consumption was reduced in high-dose rats through the first three days of dosing, though with subsequent recovery. In addition, this group displayed a reduction in body weight, marked neurological signs and 24% (6/25) lethality. However, no changes in reproductive parameters were noted; there were no soft tissue or skeletal anomalies due to RDX exposure. Dosing pregnant New Zealand white rabbits at the same levels resulted in few changes in reproductive parameters but a catalogue of teratological responses that were essentially sporadic and therefore of uncertain significance. These responses included spina bifida, misshapen cranium, meningocele, misshapen and enlarged eye bulges, abdominal wall defects, gastroschisis, appendicular reduction anomalies and "tail problems."

Cholakis et al. (1980) also reported a two-generational reproductive study in which male and female CD rats were fed diets adjusted to nominal daily doses of 0, 5, 16 or 50 mg RDX/kg for 13 weeks. F_o adults were then mated within the groups with 26 of the resulting F_1 progeny maintained on the same diets for another 13 weeks. After a further round of mating, the F_2 progeny were necropsied and processed for histopathological examination.

There was a reduction in body weight gain in all generations of high-dose rats, which may have been related to a concomitant depletion in food consumption. Mortality reached 18% in high-dose rats of the F_0 generation with 17% and 52% stillbirths in the F_1 and F_2 high-dose progeny, respectively. Reductions in the number of fertile high-dose male and female rats were observed during the F_0 mating, although these differences were statistically insignificant. Notwithstanding these changes, there appeared to be no specific reproductive or developmental changes due to treatment in this experiment, since feeding 16 mg/kg-day produced no apparent effects.

2.3.1.6 Studies Relevant to Mammalian TRV Development: RDX Ingestion Exposures

The range of animal models in which responses to acute, subacute and subchronic RDX administration have been monitored includes beagle dogs, cyanomolgus (rhesus) monkeys, Sprague Dawley and F344 rats, Swiss-Webster and B6C3F1 mice, miniature swine and New Zealand white rabbits.

There is a striking contrast between the acute lethality of RDX in experimental animals and those of other explosive/energetic compounds such as TNT and HMX. For example, acute oral LD_{50} values for the latter compounds may be found in the 500–1000 mg/kg-day range, suggesting low-to-moderate lethality, whereas the LD_{50} for RDX is in the 50–200 mg/kg-day range, with a median value closer to 100 mg/kg-day. This suggests that RDX has a higher acute toxicity than other explosive compounds. However, if RDX is characterized by comparatively high acute toxicity, the precise targets for these toxic effects remain to be fully identified. Toxicokinetic evidence indicates that the compound is readily absorbed at the gastrointestinal brush border but has a transitory existence in the body with rapid breakdown into a range of metabolic products including single carbon compounds occurring in the liver in some animals. The importance of the liver in response to RDX is underscored by the histological changes that take place when a receptor is challenged with the compound. Perturbations of clinical chemistry parameters potentially related to liver function, such as plasma lipid levels and enzyme activities such as serum glutamate-pyruvate transaminase, lend further weight to the concept that the liver is one of the primary sites of RDX toxicity.

Liver effects are also evident in a number of subchronic and chronic studies on RDX, the responses manifesting in dose-dependent increases in organ to body weight ratios and in changes to the cellular architecture revealed histologically. In the 24-month dietary study of RDX in B6C3F1 mice, histopathological evidence of compound-related hepatocellular adenoma and carcinoma formation was obtained in females. However, no effects were seen in male mice or either sex rats suggesting the response is not generally associated with rodent exposure to RDX. The ecological relevance of RDX-induced liver toxicity is questionable.

Other reasonably consistent responses that have been elicited in experimental animals exposed to RDX include changes in the levels of some hematological parameters associated with anemia and changes to the size and histopathology of the spleen. Although increased pigmentation of the spleen was used as the basis for a NOAEL of 0.30 mg/kg-day (Levine et al., 1983), associated hematological parameters, although significantly different than controls for the high dose group, fell within normal ranges (Wolford et al., 1986). This indicates that the increased pigmentation of the spleen was not associated with any hematological changes that would cause functional impairment. Given the lack of biological significance in this effect, increased pigmentation of the spleen is of questionable relevance.

As outlined in Technical Guide 254 (USACHPPM, 2000), TRVs are derived from toxicological effects likely to be ecologically relevant. Decreased growth is regarded as an ecologically relevant parameter and was common to two studies on chronic ingestion of RDX, one on F344 rats (Levine et al, 1983) and one on B6C3F1 mice (Lush et al., 1984) and two studies on subchronic ingestion of RDX, one on F344 rats (Cholakis et al., 1980) and one on Swiss Webster mice (Dilley et al., 1978). These data suggest that reduced growth is a consistent feature of RDX-exposed rodents. From an ecological perspective, reduced growth and /or associated reductions in food consumption can affect the ecological performance of individuals by causing alterations in energy allocation patterns that could ultimately result in altered reproductive performance (Calow, 1991; Congdon et al., 2001). All three studies showing reduced growth in RDX-exposed rodents were well designed and well executed and can be considered high quality. For derivation of the TRV, the data on chronic toxicity in F344 rats (Levine et al., 1983) is most appropriate as these data meet the requirements of TG 254 (USACHPPM, 2000) and as such, require no uncertainty factors. Moreover, these data are protective of the data on B6C3F1 mice (Lush et al., 1984).

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Angerhofer et al. (1986)	Rat (f) (Sprague-Dawley)	GD 6–15	NA	2	Comparative reductions in fetal size
Cholakis et al. (1980)	Rat (f) F344)	GD 6–19	2	20	Neurological signs/lethality
	Rabbit (f) (NZ white)	GD 7–29	20	NA	Reproductive/Developmental toxicity
Litton Bionetics (1974a)	Dog (Beagle)	90-d	10	NA	NA
	Monkey (rhesus)	90-d	1	10	Elevated megakaryocyte count
Brown (1975)	Rat (strain unstated)	12-w	0.3	2.5	Increased brain monoamine oxidase and cholinesterase activity
Levine et al. (1981)	Rat (F344)	90-d	30	100	Increased liver weight
McPhail et al. (1983)	Rat (m) (Sprague-Dawley)	30-d	10	NA	Neurological testing
Cholakis et al. (1980)	Rat (F344)	90-d	26.4	37.7	Reduced hemoglobin and hematocrit. Reduced body weight.
	Mice (B6C3F1)	90-d	145 (TWA)	277 (TWA)	Lethality and neurological signs, enlarged liver and hepatocellular lesions
Levine et al. (1990)	Rat (F344)	90-d	NA	5.0/29.8*	Reduced body weight gain in males
Hart (1976)	Rat (Sprague-Dawley)	104-w	10	NA	NA
Levine et al. (1983)	Rat (F344)	104-w	8	39.8	Decreased body weight
Lish et al. (1984)	Mouse (B6C3F1)	104-w	7	35	Atrophy of the testis in males, increases in relative and absolute kidney and liver weights, decreased body weight
			NA**	NA**	Hepatocellular adenoma and carcinoma in females

* Doses are those of TNT/RDX mixed in various proportions

** Identifying a NOAEL for tumorigenic responses may be unsafe, in line with existing U.S. EPA understandings on the identification of a subthreshold dose for a carcinogenic effect

GD = gestation day

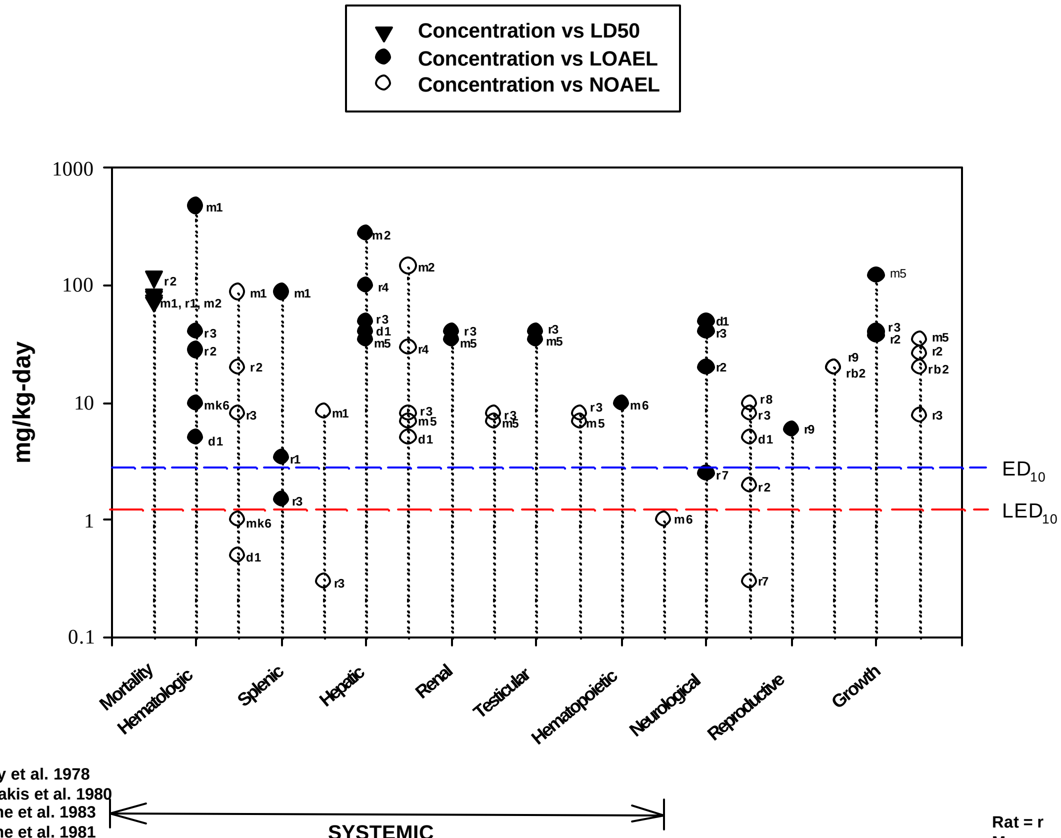
TWA = time-weighted average

NA = not applicable

RBC = red blood cell

Figure 1.

RDX HEALTH EFFECTS TO MAMMALS



HEALTH EFFECTS

1 = Dilley et al. 1978
2 = Cholakis et al. 1980
3 = Levine et al. 1983
4 = Levine et al. 1981
5 = Lish et al. 1984
6 = Litton Bionetics 1974b
7 = Brown 1975
8 = MacPhail et al. 1985
9 = Angerhofer et al. 1986
10 = Levine et al. 1990

Rat = r
Mouse = m
Dog = d
Rabbit = rb
Monkey = mk

2.3.2 Mammalian Inhalation Toxicity

No inhalation studies conducted using mammals were found.

2.3.3 Mammalian Dermal Toxicity

No dermal studies conducted using mammals were found.

2.4 Summary of Avian Toxicology

2.4.1 Avian Toxicity - Oral

2.4.1.1 Avian Oral Toxicity - Acute

One study was located on the toxicity of RDX to an avian species. Gogal et al. (2001) studied the acute, subacute, and subchronic toxicity of RDX to the Northern Bobwhite (*Colinus virginianus*). For the acute study, the objective was to determine the approximate lethal dose (ALD). RDX was administered to birds orally in a water vehicle. One male and one female per group were dosed with one of the following, 125, 187, 280, 420, 630, 945, 1417, or 2125 mg RDX/kg. Birds were observed for 14 days after administration of RDX and on day 14, surviving birds were weighed, bled, euthanized by electrocution and necropsied. The ALD values 14 days after the exposure were 280 mg/kg for male and 187 mg/kg for female Northern Bobwhite.

2.4.1.2 Avian Oral Toxicity - Subacute

Groups of six male and six female birds were exposed to RDX in the feed at concentrations of 0, 83, 125, 187, 280 and 420 ppm RDX for 14 days (Gogal et al., 2001). Daily doses of RDX were calculated to be 10.8, 13.4, 22.3 and 26.3 mg RDX/kg body weight, respectively. Feed consumption, body weight, spleen weight/body weight ratio, liver weight/body weight ratio and egg production were measured. Hematological analyses included whole blood cellularity, packed cell volume (PCV), total protein and mean corpuscular volume (MCV). Histological analyses were conducted on liver, kidney, spleen, brain, spinal cord, intestine, heart, lung, pancreas and gonad tissues.

Results showed that there was a significant, linear decrease in feed consumed with increasing levels of dietary RDX and a concomitant decrease in body weight with increasing levels of RDX in the diet. The ratios of spleen weight/body weight in females and liver weight/body weight in both sexes were also significantly affected by dose and generally decreased with increasing RDX. Hematological effects of RDX exposure included an increase in packed cell volume in females, a decline in total plasma protein in females, an increase in heterophils and an increase in the heterophil/lymphocyte ratio in blood. Egg production showed a significant, linear decrease with increasing RDX for both week one and week two.

The authors report a NOAEL of 8.7 mg RDX/kg/day and a LOAEL of 10.6 mg RDX/kg/day based on a dose-related decrease in body weight and egg production.

2.4.1.3 Avian Oral Toxicity - Subchronic

Five groups of 10 male and female Northern Bobwhite were provided with 0, 125, 187, 280 or 420 ppm RDX in the feed for 90 days (Gogal et al. 2001). The calculated daily oral doses were reported to be 0, 10.8, 13.4, 22.3 and 26.3 mg/kg for the 0, 125, 187, 280 and 420 ppm, respectively. Feed was weighed and replaced on a weekly basis. Parameters measured included those mentioned in the 14-day study including 5-part leukocyte differentials, lymphocyte mitogen-induced proliferation and leukocyte apoptosis/necrosis assays. Histological analyses were as above with the addition of bone marrow. Changes in egg production were also evaluated. Although the same doses used in the subacute were identical to those in the subchronic study, no significant effects of RDX were seen after exposure for 90 days. These data suggest that Northern Bobwhite develop a tolerance from exposure to RDX in the feed. However, although no significant effects were seen, there were dose-dependent trends apparent for several parameters including, a decrease in feed consumption, decrease in total protein, a decrease in PCV and a decrease in egg production. No severe effects were noted. Since no significant effects of RDX were seen after 90 days of exposure, a LOAEL was not reported.

2.4.1.4 Avian Oral Toxicity - Chronic

No data are available.

2.4.1.5 Avian Oral Toxicity - Other

No data are available.

2.4.1.6 Studies Relevant for Avian TRV Development for Ingestion Exposures

Only one study was located on the effects of RDX on an avian species. Gogal et al. (2001) investigated acute, subacute, and subchronic effects of orally administered RDX in Northern Bobwhite (*Colinus virginianus*). In the 14-day study, there were significant effects of RDX on both body weight and egg production. In the 90-day study, the same doses of RDX were used as in the 14-day study, however, no significant effects of RDX were seen, although there were dose-dependent decreases in body weight and total egg production. These data suggest that Northern Bobwhite develop a tolerance to prolonged dietary exposure to RDX. Although data from long-term exposures (i.e., subchronic and chronic) are preferred, in this case the subacute data on egg production is especially relevant. A rationale is provided below.

Birds are highly vagile animals and thus often experience the environment in patchily distributions. Under these realistic exposure scenarios, birds are most likely to experience short-term exposures on the order of days as opposed to weeks. Therefore, a 14-day exposure to RDX may be more ecologically relevant than longer exposure scenarios. Moreover, these data are protective of longer exposure scenarios tested to date. Although these changes in egg production and other parameters (e.g., body weight gain) may be due to the reduction in consumed feed, food avoidance may also be an ecologically relevant parameter. Since the primary endpoint chosen is a reproductive one, under TG 254 and consistent with Sample et al. (1996), data on egg production in quail exposed to RDX for 14 days can be considered equivalent to a long-term investigation since the exposure occurred during a sensitive life cycle stage. Hence, the avian TRV for RDX was derived from the 14-day oral exposure in Northern Bobwhite (Gogal et al., 2001).

Table 3. Summary of Relevant Avian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL mg/kg/d	LOAEL mg/kg/d	Effects at LOAEL
Gogal et al. (2001)	Northern Bobwhite (*Colinus virginianus*)	ALD	NA	NA	187 mg/kg for female 280 mg/kg for male
		14 d	8.7	10.6	Decreased body weight in males and females and decreased egg production.
		90 d	26.3	NA	No statistically significant effects however, there were several dose-related trends; decreased egg production, feed consumption, total plasma protein and packed cell volume.

ALD = approximate lethal dose
NA = not applicable

2.4.2 Avian Inhalation Toxicity

No data are available.

2.4.3 Avian Dermal Toxicity

No data are available.

2.5 Amphibian Toxicology

Toxicological studies on the effects of RDX in amphibian species were not located. Ecotoxicological research on the effects of RDX on amphibians is recommended.

2.6 Reptilian Toxicology

Toxicological studies on the effects of RDX in reptilian species were not located. Ecotoxicological research on the effects of RDX on reptiles is recommended.

3 RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Decreased body weight was reported for both F344 rats (Levine et al., 1983) and B6C3F1 mice (Lish et al., 1984) after two years of oral dosing with RDX. Decreased body weight, an indication of a lower growth rate or a decrement in energy allocation, was used to determine the TRV because this endpoint may be ecologically relevant through effects on fitness. For example, indicated alterations in energy allocation patterns may impair reproductive function and/or schedules (Calow, 1991; Congdon et al., 2001). In addition, sustaining a smaller body size for longer time periods may increase risk of predation. Both chronic studies (Levine et al., 1983; Lish et al., 1984) indicated decreased growth in rats and mice fed RDX, and hence the effect may be a consistent feature of RDX exposure. For TRV determination, data on female F344 rats was used because these data were protective of males and exhibited a clear dose response relationship (Levine et al., 1983). In addition, the TRV based on the F344 rat data was protective of B6C3F1 mice. Growth, as indicated by body size, also meets the minimum data requirements of the Standard Practice, Section 2.2 (USACHPPM 2000) and therefore no uncertainty factors were required in the derivation of the TRV. The TRV was derived using the Benchmark dose approach (Appendix B) and the values presented in Table 4. This TRV is given a medium confidence rating since there were only two chronic studies and no wildlife toxicity data were available.

Table 4. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
LED_{10}	1.19 mg/kg/d	Medium
ED_{10}	2.73 mg/kg-d	Medium

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not Available at this time.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time

3.2 Toxicity Reference Values for Birds

3.2.1 TRVs for Ingestion Exposures for the Class Aves

The ecologically relevant parameter for RDX toxicity in birds was decreased fecundity (i.e. egg production) reported for Northern Bobwhite exposed to dietary concentrations of RDX for 14 days (Gogal et al., 2001). For this endpoint, the effect was significant and dose dependent and the study was of high quality. Decreased egg production was used to determine the TRV because it is an ecologically relevant parameter indicative of impaired reproductive performance, which can have direct impacts on population dynamics, particularly for this species.

Exposure to RDX in this study occurred during a sensitive life cycle stage, and therefore can be considered equivalent in value to a chronic exposure evaluation. Given the data quality, the dose dependent nature of the effect, and the ecological relevance of effect, the Benchmark Dose approach was used. The TRVs derived using the Benchmark dose approach (Appendix C) are presented in Table 5. It should be noted that although there was not a significant effect of RDX on egg production in quail for the 90-day exposure, there was a trend; egg production decreased with increasing concentrations of RDX. The Benchmark Dose approach was applied to these data as well and are presented and Appendix D. A benchmark dose (BMD or ED_{10}) of 8.14 mg/kg-d was calculated from the model fit of the mean response at the 10% response level. A lower-bound on the benchmark dose (BMDL or LED_{10}) was calculated to be 3.65 mg/kg-d from the lower 95% confidence interval (CI) of the modeled curve. Comparison of Benchmark Doses for the 14-day and 90-day studies indicate that TRVs derived from the 14-day study are protective of TRVs derived from the 90-day study. Since data from only one study was located, the TRVs presented below are given a low confidence rating.

Table 5. Selected Ingestion TRVs for the Class Aves

TRV	Dose	Confidence
LED_{10}	3.65 mg/kg/d	Low
ED_{10}	8.14 mg/kg-d	Low

3.3 Toxicity Reference Values for Amphibians

Not Available at this time.

3.4 Toxicity Reference Values for Reptiles

Not Available at this time.

4. IMPORTANT RESEARCH NEEDS

The limited availability of data on the toxicity of RDX to wildlife species precludes the development of a high-confidence TRV. Hence, more studies on the toxicity of RDX to wildlife species are needed. In particular, long-term toxicity studies on mammals and additional studies on non-mammalian wildlife such as birds, reptiles and amphibians are particularly warranted. More information regarding the toxicity of RDX to wildlife would likely allow the derivation of a high confidence TRV.

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APPENDIX A
LITERATURE REVIEW

The following files were searched in Dialog:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 10 AGRICOLA, File 203 AGRIS, File 399 Chemical Abstracts, File 337 CHEMTOX, File 77 Conference Papers Index, File 35 Dissertation Abstracts, File 40 ENVIRONLINE, File 68 Environmental Bibliography, File 76 Life Sciences Collection, File 41 Pollution Abstracts, File 336 RTECS, File 370 Science, File 143 Wilson Biological & Agricultural Index, File 185 Zoological Record, File 6 NTIS, File 50 CAB, File 144 PASCAL, File 34 SCISEARCH.

The search strategy for **Amphibians & Reptiles**:

- Chemical name, synonyms, CAS numbers
- AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- RD (reduce duplicates)

The search strategy for **Birds:**

- Chemical name, synonyms, CAS numbers
- And chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- RD

The search strategy for **Laboratory Mammals:**

- Chemical name, synonyms, CAS numbers
- AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de
- NOT ((meeting()poster) or (meeting()abstract))
- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- RD

The search strategy for **Wild Mammals:**

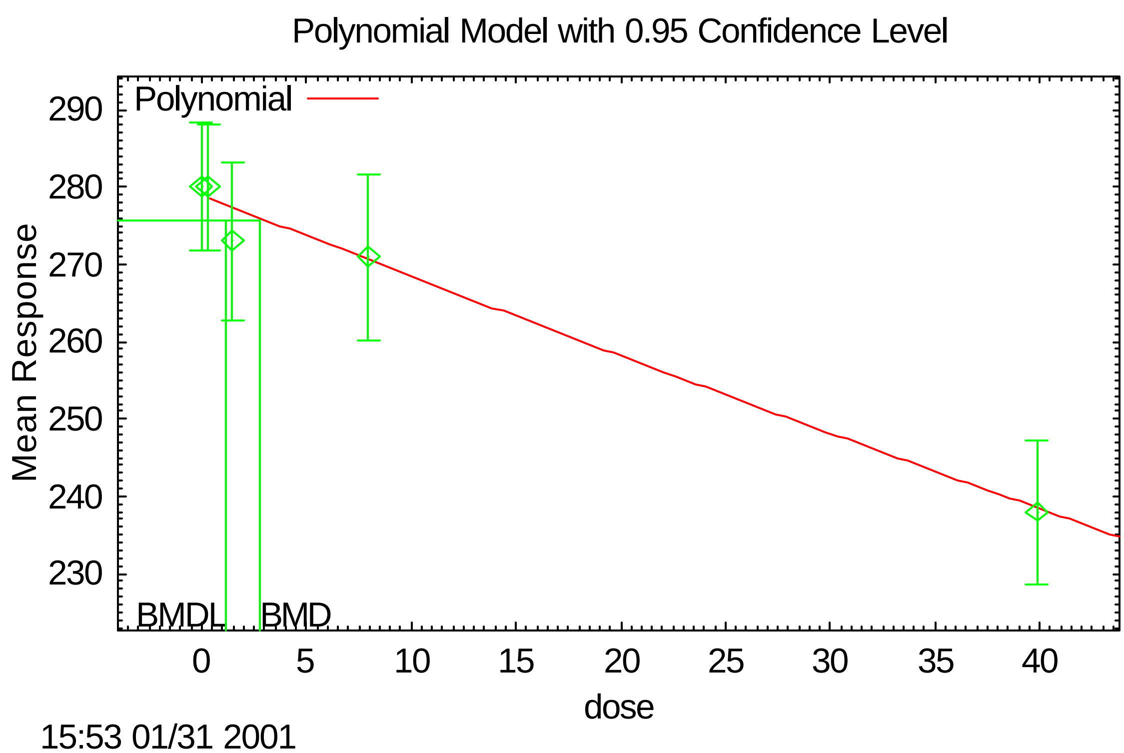
- Chemical name, synonyms, CAS numbers
- And(didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 31 hits on RDX were obtained in the initial search, all of which were selected for abstract evaluation. Nineteen of these articles and reviews were retrieved for this survey.

APPENDIX B
Benchmark Dose Calculation for Mammals

The data presented below are from Levine et al. (1983) with mean body weight at two years in Fischer 344 rats as the response. Data from females was used since it showed a clear dose response and was protective of males. The model fit was adequate, and a benchmark dose (BMD) and benchmark dose low (BMDL) were obtained from this analysis.



The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	868.792
beta_0 =	278.559
beta_1 =	-1.05871
beta_2 =	0.00104466

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	851.428	0.0116566
beta_0	278.62	0.481016
beta_1	-1.07224	7.18802
beta_2	0.00135624	277.868

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	5.8e-007	4e-007	-5.8e-007
beta_0	5.8e-007	1	0.49	0.39
beta_1	4e-007	0.49	1	0.98
beta_2	-5.8e-007	0.39	0.98	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
------	---	--------	-----------	--------	-----------	----------
0	43	280	27	279	29.2	0.0473
0.302	45	280	27	278	29.2	0.0584
1.486	42	273	33	277	29.2	-0.138
7.969	41	271	34	270	29.2	0.0287
39.85	26	238	23	238	29.2	-0.00151

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-762.527	6	1537.05
A2	-758.957	10	1537.91
fitted	-763.071	4	1534.14
R	-781.587	2	1567.17

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	45.2597	8	<.0001
Test 2	7.14095	4	0.1286
Test 3	1.0875	2	0.5806

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.950000

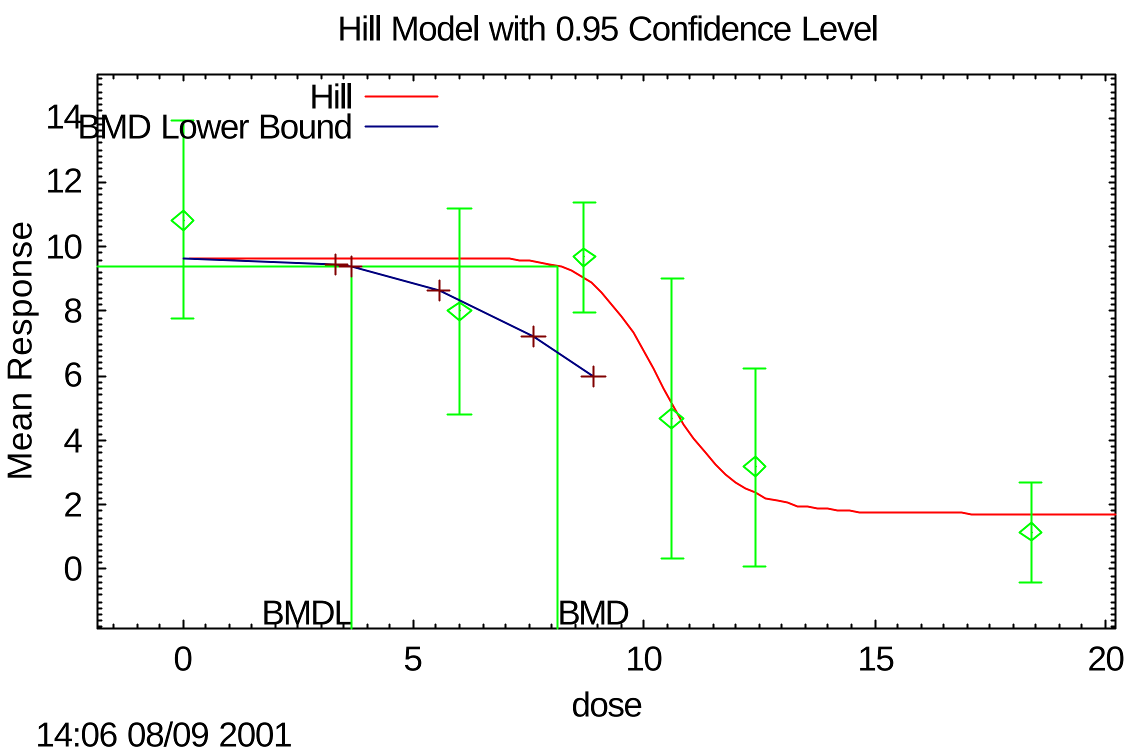
BMD = 2.73077

BMDL = 1.18567

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted

APPENDIX C
Benchmark Dose Calculation for Bobwhite Quail

The data presented below are total egg production from quail exposed to RDX in the feed for 14 days from Gogal et al. (2001). These data were considered for the TRV because they represent a sensitive stage in the life cycle and are protective of 90-day effects. The model fit was adequate, and a benchmark dose (BMD) and benchmark dose low (BMDL) were obtained from this analysis.



The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 6
Total number of records with missing values = 0

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	6.36633
rho =	0 Specified
intercept =	10.83
v =	-9.663
n =	8.29994
k =	10.0943

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	intercept	v	n	k
alpha	1	0	0	0	0	0
rho	0	1	0	0	0	0
intercept	0	0	1	0	0	0
v	0	0	0	1	0	0
n	0	0	0	0	1	0
k	0	0	0	0	0	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	7.65144	1
rho	0	1
intercept	9.6589	1
v	-7.94406	1
n	13.7149	1
k	10.3768	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	6	10.8	2.93	9.66	2.77	0.423
6	6	8	3.03	9.65	2.77	-0.598
8.7	6	9.67	1.63	9.01	2.77	0.239

10.6	6	4.67	4.13	5.11	2.77	-0.161
12.4	6	3.17	2.93	2.35	2.77	0.295
18.4	6	1.17	1.47	1.72	2.77	-0.199

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-52.235092	7	118.470185
A2	-48.036623	12	120.073247
fitted	-54.628079	5	119.256157
R	-71.471672	2	146.943344

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	46.8701	10	<.0001
Test 2	8.39694	5	0.1357
Test 3	4.78597	2	0.09136

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears
to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

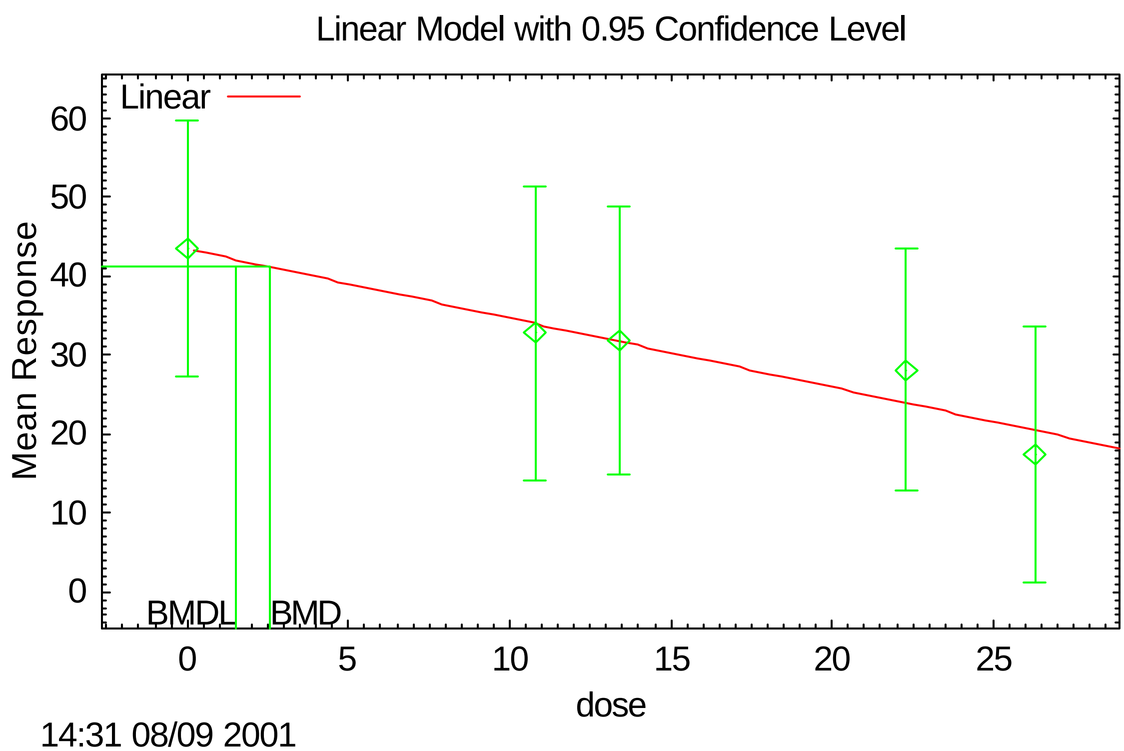
Confidence level = 0.95

BMD = 8.14449

BMDL = 3.6501

APPENDIX D
Benchmark Dose Calculation for Bobwhite Quail

The data presented below are total egg production from quail exposed to RDX in the feed for 90 days from Gogal et al. (2001). These data were not significant, however, a dose-related trend is readily apparent. The model fit was adequate, and a benchmark dose (BMD) and benchmark dose low (BMDL) were obtained from this analysis, although the approximations are suspect due to the lack of significance in the effect.



The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	544.388	
rho =	0	Specified
beta_0 =	43.4465	
beta_1 =	-0.874347	

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	495.548	99.1096
beta_0	43.4465	5.88362
beta_1	-0.874347	0.341382

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1
alpha	1	-2.4e-007	-2.5e-008
beta_0	-2.4e-007	1	-0.84
beta_1	-2.5e-008	-0.84	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
26.3	10	17.4	22.5	20.5	22.3	-1.37
22.3	10	28.1	21.4	23.9	22.3	1.86
13.4	10	31.8	23.8	31.7	22.3	0.0224
10.8	10	32.8	26.1	34	22.3	-0.541
0	10	43.5	22.6	43.4	22.3	0.024

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2
Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-179.857527	6	371.715054
A2	-179.628138	10	379.256275
fitted	-180.141630	2	364.283260
R	-183.728560	2	371.457121

Test 1: Does response and/or variances differ among dose levels
(A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	8.20085	8	0.08449
Test 2	0.458779	4	0.9774
Test 3	0.568206	3	0.9037

The p-value for Test 1 is greater than .05. There may not be a diffence between responses and/or variances among the dose levels. Modelling the data with a dose/response curve may not be appropriate

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 2.546

BMDL = 1.53076

BMDL computation failed for one or more point on the BMDL curve.
The BMDL curve will not be plotted